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(54) **Plant Extract Disinfectant**

(57) A non-toxic disinfectant comprises one of its major ingredients the Wu-ren solution which is a mixture of extracts from various Chinese medicinal plants. This solution which is antimycotic, antibacterial, and antimicrobiological contains such bactericidal compounds as oleanolic acid, ursolic acid, fumaric acid, benzene-carboxy lic acid and succinic acid as the main active ingredients. The disinfectant is non-toxic and hypoallergenic. The solution is effective for concentrations ranging from 1 to 100 percent.

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Title : A Non-toxic Disinfectant

This invention relates to a disinfectant, particularity shows excellent antimyeotic and anti-microbiological effects.

5 There are many types of harmful microbiological agents in the air that could enter the human body through breathing. Depending on the physiological condition of the individual, each exposure to such agents will result in different degrees of physiological and pathological reactions.

10 A general survey shows that there are about forty one major types of infections diseases in the world that are spread through various media. About fourteen of which are spread through the air making it the number one medium. During the 1980's a survey of infections diseases conducted in the United Kingdoms showed that respiratory tract
15 infection ranks as the number one infections disease consisting of 30.3% of total number of contractions. At the same time, the problems of secondary infection and the prevention of the spread of infections diseases in the hospitals, have remained important issues. For these reasons
20 a great deal of international attention is focused on the methods of effectively cleaning and disinfecting the air. Various countries have already under taken major R & A efforts in finding different kinds of disinfectants in the attempt to reduce the health hazards caused by air-borne
25 microbiological agents. Presently there are several scores of commercially available disinfectants on the market. Although

they are generally effective as aerial and surface disinfectants, they are nevertheless toxic, irritative and corrosive and tend to have environmental problems that limit their application in hospitals, drug manufacturing plants and food processing factories.

The present invention concerns a non-toxic disinfectant of which the Wu-ren solution is the major effective ingredient. This disinfectant shows excellent antimyeotic, antibacterial and anti-microbiological effects and is effective in the treatment of skin diseases, psoriasis, beriberi and hepatitis.

Recently, a novel disinfectant has been developed from extracts of certain natural plants. We call it the Wu-ren solution. It is not only non-toxic but also highly effective in destroying various kinds of hazardous microbes and could be safely utilized in our living environment.

The Wu-ren solution which is separated and extracted from various medicinal plants is clearly effective in suppressing and/or destroying various bacteria, fungi and viruses. After proper processing, it can be used as a food preservative for as well as a disinfectant or a curative medicine. Since it is a natural substance high in its disinfectiveness and low in its toxicity, its commercial value is considerable.

Through extraction, separation by photo-chromatography and GC/MS analysis the main chemical compounds of the Wu-ren

solution are identified as follows:

- (1) Aalkyls: such as toluene, ethyl-benzene, indene which possess antibacterial characteristic.
- (2) Polyakyl aldehyde and its esters: such as octanoic acid, methyl hexadecanate which have a strong antibacterial characteristic as well as an anti-cancer characteristic.
- (3) Phenols: which promote the settlement of protein, could change the permeability of cell membrane. And have strong antibacterial and preservational characteristics.
- (4) Benzoic acids: which are effective against food deterioration and bacterial infection.
- (5) Acids: such as oleanolic acid, ursolic acid, fumaric acid, succinic acid and benzene-carboxylic acid which are all excellent in disinfecting against bacteria.
- (6) Other: such as furans which can suppress the actions of acetylase and interfere with the metabolism of bacteria; or pyridine which can also destroy germs through the disruption of their metabolism.

Three types of Wu-ren solutions were prepared and are designated A1, B1, and B2. A1 is a white, transparent and water soluble solution which is slightly acidic B1 and B2 are brown, oily solutions which have a burnt smell and are soluble in such organic solutions as ethanol and ethyl

acetate. Its specific gravity is 1.027 at 10 C and have a pH value between 5 to 6.

The toxicity and the bactericidal quality of the Wu-ren solutions were demonstrated by the following experiments. These experiments are introduced in order to demonstrate the effectiveness of the Wu-ren solution in the destruction of microbiological agents and should not be construed to prescribe a limit to the use of the solution in the present patent application.

Example 1, acute toxicity test

(1) Twenty mice were selected 50% of them were male and 50% female. Their body weights range between 17 to 20 g. The mice were separated into two groups. One group was forced fed directly to the stomach of A1 solution at 0.5 ml per 20g body weight. The other group was given B1 solution also at 0.5 ml per 20 g body weight. Observations were made continually for one week after administration of the solution. None of the mice died as a result of this experiment. (Room temperature was set at 15 C.)

(2) Four healthy domesticated rabbits were selected, each weighing approximately 2 kg. Three rabbits were forced fed directly to the stomach of A1, B1 and B2 solutions respectively at 10 ml per 2 kg body weight. The fourth was given 10ml per 2kg of saline. Observations were made continually for one week after administration of the

solutions. None of the rabbits died or exhibited symptoms of intoxication. The dosage involved in the experiment were directly administered to the stomach and was equivalent to 5000 ml/kg. And was equivalent to three times the dosage of administered orally (LD50 1500mg/kg.)

Example 2, LD50 oral dosage test

Fifty mice were selected. 50% of them were male and 50% female. They were further separated into 5 groups and fed by normal method diluted solution through the stomach. After the dosages were given, the mice were observed for a week afterwards and the condition of death were recorded and the bodies dissected. Bliss calculation showed that LD50 is equivalent to about 1737.90 mg/kg with 95% of LD50 between 1399.6 to 1689.6 mg/kg. From these results, it was concluded that Wu-ren solutions are lower in toxicity then phenols (LD50 = 282 mg/kg) or benzoic acid. All death occurred 24-hours after being fed the solution, and the mice appeared normal at the time. Their tissues after dissecting do not show any apparent pathological changes.

Example 3, skin irritation test

Twenty four brown rats with weights between 250 to 350g were selected. Half of them were male and other half female. They were evenly divided into 4 groups with testing solutions of A1, B1, B2 and a control solution. Skin irritation tests were carried out according to the DECD method. Skin reactions

such as edema, erythema were observed one hour, 24 hours, 48 hours and 72 hours after the solutions were applied to the skin.

The degrees of skin reaction were evaluated as follows:

Degree of reaction	Ratio	
None	0.0	0.4
Slight	0.5	1.9
Medium	2.0	5.9
Strong	6.0	8.0

Skin tests at room temperature and 73% humidity showed the following results:

Group	Erythema(%)	Edema(%)	Total	Degree of Stimulation
A1	0.16	0	0.16	none
B1	0.33	0	0.33	none
B2	0.5	0.16	0.66	slight
control	1.33	0.5	1.83	slight

From these test data, it could be concluded that Wu-ren solutions A1 and B1 are almost totally non-irritating and B2 and the control solution are only slightly irritating.

Example 4, disinfection tests of Wu-ren solutions

I. Results of disinfection tests against Staphylococcus

aureus, Escherichia coli, Shigella dysenteriae, and Pseudomonas aeruginosa with the Wu-ren solution according to the MBC method were recorded.

Concentration	Bacterial Sol (8 x 10/ml)	Solution (ml)	Dosage of the disinfection sol(ml)
1%	0.5	1.40	0.02
5%	0.5	1.40	0.10
10%	0.5	1.30	0.20
15%	0.5	1.20	0.30
20%	0.5	1.10	0.40

Test results showed:

- (1) A1 and B2 at 15% concentration were effective in disinfecting E. coli, S. dysenteriae and P. aeruginosa within ten minutes.
- (2) B1 at 15% concentration disinfected E. coli, S. dysenteriae k and P. aeruginosa within only five minutes.
- (3) B1 at 20% concentration disinfected S. aureus within five minutes.

II. Wu-ren solutions were tested against Serratia Marcescens with the test tube method. Results are shown as follows:

	Disin- fectant	Concen- tration(%)	Average live bacteria count	Bactericidal rate	t	p
		100	13.0	79.03	13.57	<0.001
	A1	50	14.0	82.26	15.37	<0.001
5		1	18.0	70.97	14.31	<0.001
	B1	100	0.7	98.87	19.04	<0.001
		50	11.3	81.77	19.20	<0.001
		1	20.0	67.74	8.09	<0.05
		0	62.0			
10		100	0.0	100	155.41	<0.001
	B2	50	0.3	99.48	98.62	<0.001
		1	37.0	35.65	6.74	<0.05
		0	57.5			

III. Wu-ren solutions were tested against Bacillus subtilis
 using test tube method. Results are shown as follows:

	Disin- fectant	Concen- tration(%)	Average live bacteria count	Bactericidal rate(%)	t	p
		100	39.3	39.26	4.99	<0.01
	A1	50	47.7	26.29	3.67	<0.05
20		1	51.0	21.17	2.24	<0.05
		0	64.7	0.00		
		100	16.3	67.4	9.01	<0.001
	B1	50	31.0	38.0	3.71	<0.05
		1	44.0	11.4	1.52	<0.05
25		0	50.0	0.0		
		100	1.70	97.21	41.70	<0.001
	B2	50	8.70	85.74	28.60	<0.001

1 64.00 0.00 0.78<0.0

IV. Wu-ren solutions were tested against Mycobacterium tuberculosis using the test tube method. After two repeated tests, results showed that 100% concentration of A1, B1 and B2 were added when to 10 to 10 concentration of M. tuberculosis solution, the M. tuberculosis ceased growing after five minutes.

V. Wu-ren were tested against Neisseria gonorrhea using the test tube method. Test results showed that 100% concentration of A1 solutions is effective in disinfecting against the germ within five minutes and 50% concentration of A1 solution is effective within ten minutes (no bacterial growth).

Example 5, disinfection of Wu-ren solutions against mold and mildew

Wu-ren solutions were tested against Candida albicans and Aspergillus flavus. Results are shown as follows:

Disinfectant	Concentration	Average live bacterial count		Bactericidal rate (%)	
		<u>C. albicans</u>	<u>A. flavus</u>	<u>C. albicans</u>	<u>A. flavus</u>
A1	100	0.0	0.0	100.0	100.0
	50	7.0	0.0	91.7	100.0
	25	7.7	7.0	90.2	93.0
	10	54.7	92.0	31.0	8.0
	1	70.4	94.0	6.7	6.0
	0	79.3	100.0	0.0	0.0

	100	0.0	0.0	100.0	100.0
	50	0.0	0.0	100.0	100.0
B1	25	0.6	7.0	99.3	93.0
	10	1.0	96.0	98.7	4.0
	1	48.0	102.0	39.5	0.0
	0	79.3	100.0	0.0	0.0

	100	0.0	0.0	100.0	100.0
	50	0.0	0.0	100.0	100.0
B2	25	0.0	0.0	100.0	100.0
	10	0.3	0.0	99.6	100.0
	1	4.7	45.0	94.1	100.0
	0	79.3	100.0	0.0	0.0

Wu-ren solution A1 at 30% concentration showed excellent disinfecting effects against Aspergillus flavus, A. niger, Penicillium, Trichoderma, Nigrospora, etc. as tested by using the MBC method.

Example 6, Wu-ren solutions were tested against virus

I. Wu-ren solutions were tested against bacteriophage T-3.

The results are shown as follows: (within 10 minutes)

Disin- fectant	Concen- tration(%)	Average live bacteria count (plaque)	Bactericidal rate(%)
	100.0	0.0	100.0
A1	50.0	0.0	100.0
	10.0	25.0	96.7
	1.0	79.0	86.1
	0.0	760.0	

	100.0	0.0	100.0
	50.0	0.0	100.0
B1	10.0	71.0	90.0
	1.0	88.0	88.4
	0.0	760.0	
	100.0	0.0	100.0
B2	50.0	0.3	99.9
	10.0	54.0	92.9
	1.0	72.0	90.5
	0.0	760.0	

II. Wu-ren solutions were tested against HBsAg for disinfection by using the SPRIA test method.

Take 0.1 ml of HBsAg positive serum (RPHA method 1:2048), then 0.4 ml of different concentrations of Wu-ren solutions of A1, B1 and B2 types were added. Each concentration of solution was tested for 2, 5 and 10 minutes before being neutralized by the addition of a neutralizing agent. The mixtures were then allowed to interact for 30 minutes before the test was terminated. Standard positive and negative reactions were set with positive without adding Wu-ren solution, but saline solution was added and with negative without adding HBsAg positive serum but saline solution was added. If P/N value is less than 2.1 then it can be concluded that negative serum is effective and if P/N value is less than 72.1, it means that positive serum is not effective.

Effect of the A1 solution on HBsAg (P/N value)

	Concen- tration(%)	Time		
		2 min.	5 min.	10 min.
5	100	0.9	1.1	0.7
	50	9.9	9.9	11.6
	25	9.9	9.9	15.3

Effect of the B1 solution on HBsAg (P/N value)

	Concen- tration(%)	Time		
		2 min.	5 min.	10 min.
10	100	1.9	1.6	2.0
	50	2.6	2.6	1.8
	25	16.3	9.9	9.3

Effect of the B2 solution on HBsAg (P/N value)

	Concen- tration(%)	Time		
		2 min.	5 min.	10 min.
15	100	1.3	1.1	0.9
	50	0.9	0.9	0.7
	25	9.9	9.3	3.9

At 50 100% concentration for 5 to 10 minutes, it was effective in disinfecting HBsAg.

III. The disinfecting effect of the Wu-ren on HIV-1 and lymphocyte

25 It has been proven with experiments that Wu-ren solutions are almost completely non-toxic to lymphocyte yet were effective in destroying HIV-1. The effective concentration range was between 1:32 1:50 with the time range of 2 5 minutes.

CLAIMS

1. A non-toxic disinfectant which is characterized by the presence of the Wu-ren solution as the main active ingredient and is effective against antimycotic, antibacterial, antimicrobics with effective concentration than ranges between 1% to 100%.
2. A disinfectant as claimed in claim 1 wherein the said main acidic compounds of the Wu-ren solutions are oleanolic acid, ursolic acid, fumaric acid, hydroxy benzoic acid and succinic acid.
3. A disinfectant as claimed in claim 1 wherein the said disinfectant can be either in liquid state or in gaseous state.
4. A disinfectant as claimed in claim 1 wherein the said disinfectant can be used in hospitals, in the food industry or in pharmaceutical plants where constant human exposure is expected.

Patents Act 1977
Examiner's report to the Comptroller under
Section 17 (The Search Report)

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Relevant Technical fields

(i) UK Cl (Edition L) A5E EAB EBB

(ii) Int Cl (Edition 5) A01N

Databases (see over)

(i) UK Patent Office

(ii) ONLINE DATABASES: WPI

Search Examiner

P N DAVEY

Date of Search

14 APRIL 1993

Documents considered relevant following a search in respect of claims 1-4

Category (see over)	Identity of document and relevant passages	Relevant to claim(s)
	NONE	

Category	Identity of document and relevant passages 15.	Relevance to claim(s)

Categories of documents

X: Document indicating lack of novelty or of inventive step.

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